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CREEP FEED RATIONS FOR SUCKLING PIGS WITH ADDITIONAL STUDIES RELATING TO THE VITAMIN CONTENT OF SWINE MILK AND BLOOD PLASMA

by

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April 1955

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CREEP FEED RATIONS FOR SUCKLING PIGS WITH ADDITIONAL STUDIES RELATING TO THE VITAMIN CONTENT OF SWINE MILK AND BLOOD PLASMA

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
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by
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ABSTRACT

During 1954 an experiment was conducted to study the addition of supplemental riboflavin in the creep feed rations of swine and also to determine the effect on the palatability of such rations when Anifeed Dribase Pig Feed Flavor was included.

The rate of gain of the suckling pigs did not appear to be influenced by the supplemental riboflavin while the inclusion of the feed flavour did not appear to increase the palatability of the creep feed ration as measured by feed consumption.

Measurements of the vitamin A and riboflavin content of sows' milk indicated that there was no seasonal variation in the level of vitamin A, though there was a significant seasonal variation in the acid liberated riboflavin content of the milk.

There was a definite relationship between the vitamin A level of the blood plasma of sows and of their suckling pigs. A regression equation for the prediction of the vitamin A level of the blood plasma of suckling pigs, assuming a knowledge of the level of the vitamin in the blood plasma

of their dams, has been formulated.

Studies relating to the haemoglobin content of the blood of young pigs showed that although the level varied when different levels of reduced iron were administered, the overall difference when individual pig levels were considered did not appear to be significant. The differences in the average haemoglobin levels were probably due to the differences in the levels of iron administration, though seasonal effects could have been of major importance.

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CREEP FEED RATIONS FOR SUCKLING PIGS WITH ADDITIONAL STUDIES RELATING TO THE VITAMIN CONTENT OF SWINE MILK AND BLOOD PLASMA

INTRODUCTION

Numerous factors influence the vigour and growth of young pigs, but none is more important than the feed supply. Recent work on the artificial rearing of pigs from shortly after birth has met with varied results and does not, at present, appear to be feasible in Alberta. In consequence a greater emphasis has been placed on the study of sows' milk and creep feed rations. The work reported in this thesis was conducted from January to December, 1954.

The protein, lactose, fat and mineral content of sows' milk have been widely studied, while a certain amount of analytical work has been conducted on the vitamin content of this product. The variations in results which have been obtained from such vitamin studies are considerable and it was therefore decided to assay both vitamin A and riboflavin in samples of sows' milk collected at the University Farm. In conjunction with these milk studies the vitamin A content of the plasma of nursing sows and of suckling pigs was also measured.

Experimental work on creep feeding is somewhat limited though such practice is widely advised. To be economically

worthwhile a large proportion of such a ration should be produced locally, hence results of creep feed experiments conducted in other crop areas are of limited practical value. Work at the University of Alberta during recent years has resulted in the formulation of a creep feed ration suitable for use in this province; however, during 1954 in an effort to improve this ration an experiment was conducted in which certain alterations and modifications were made to the aforementioned ration. In order to determine whether the ration contained sufficient natural riboflavin, supplements of this vitamin were given to a portion of the litters; while in an effort to increase palatability a commercial feed flavour was included in the creep feed rations of some of the young pigs.

REVIEW OF LITERATURE

There is a lack of precise information in the literature on the nutrient requirements of pigs under eight weeks of age, as well as on the importance of levels of certain vitamins in the milk and blood of pigs. The following review summarizes the results of experiments reported in these closely related fields of research.

Creep feeding of suckling pigs

The sow is reported to reach her lactation peak approximately three weeks after farrowing, following which there is a steady decline in milk yield until weaning (Henry and Woll, 1897; Hughes and Hart, 1935; Smith, 1937; Davidson, 1949; Carrol and Krider, 1950; Bowland and McElroy, 1952; and Hammond, 1952). It is noted, by most of these workers and also by MacEwan and Ewen (1936), Linton and Williamson (1943), Halnan and Garner (1947) and Morrison (1948), that following the lactation peak, at about three to four weeks of age, suckling pigs tend to become interested in solid feed. Young pigs may start to eat the sows' feed but this should be discouraged by offering them their own source of feed in a creep. The sows' feed is usually too high in fibre to be of much value to the young pig while the balance of other nutrients is generally unsuitable. A further reason for discouraging young pigs from eating

with the sow is that when eating, the sow is liable to trample or otherwise injure the pigs.

Pigs offered creep feed have been shown to grow faster and to be weaned at heavier weights than pigs raised without this advantage (Halnan and Garner, 1947; Anderson, 1950; Waldern and Wood, 1952; Bowland, 1953; and Cunha, mimeo.). These workers also report that creep-fed pigs do not show such a marked reduction in growth rate at weaning as do non-creep-fed pigs.

Davidson (1949) reports that in his experience it is the pigs which receive the most milk which are the first to start eating creep feed. He recommends a dry creep feed for the following reasons: it may be self fed, hence allowing the pigs to eat little and often; and as it will not sour, the chances of gastric troubles caused by the ingestion of bad feed are reduced. An added advantage is the small amount of labour necessary for such management practice.

The essentials of a good creep feed ration are that it should supply all the necessary nutrients and at the same time be highly palatable. Linton and Williamson (1943) recommended a crude protein level of approximately 20% with a crude fibre of less than 3 percent. Using a synthetic diet Reber et al. (1953) determined that the baby pig required a protein level of 20% as it approached weaning. These levels compare favourably with those

successfully fed by Bowland (1953) in Alberta, who used a creep feed ration containing 79% calculated total digestible nutrients, 18% total protein (15% calculated digestible protein), with a crude fibre content of 4.5 to 5.0%, the ration being well fortified with minerals, vitamins and antibiotics.

Feed flavours in pig feeds

The use of flavour compounds to increase the palatability of feeds is a relatively recent development. The only available report of an experiment with feed flavours in the rations of young pigs was published by Hanson *et al.* from the University of Minnesota (1954). To a series of basal creep feeds 'Flavor M (molasses fortifier)' and 'Flavor A.M. (anise-molasses)' were added. On offering both basal and flavoured creep feeds free choice to several groups of young pigs it was found that they consumed approximately twice as much basal as flavoured feed. This would indicate that the pigs did not care for the flavours offered.

The unreported work of Clandinin and Robblee (1954) would indicate that 'Anifeed Dribase Poultry Feed Flavor' had no effect upon the feed consumption or rate of gain of chicks.

Riboflavin requirement of the pig

Early studies on the riboflavin requirement of the pig extend back almost 20 years. Chick and co-workers (1938) revealed the need of the pig for certain components of the vitamin B_2 complex but did not show whether riboflavin is an essential dietary constituent for the pig. Hughes (1939) observed that 'pigs fed a diet deficient in riboflavin gained very slowly, defecated frequently semi-liquid faecal material, became crippled and walked with difficulty.' In a later report Hughes (1940) estimated by means of growth curves, that the minimum daily requirement of riboflavin for the young growing pig lies between 1 and 3 mg. per 100 lb. of pig. Wintrobe (1939) likewise observed that the addition of riboflavin to a diet deficient in the B-complex vitamins accelerated the rate of growth.

Using a semi-synthetic diet, Patek *et al.* (1941) demonstrated that riboflavin is an essential dietary constituent for the pig. A deficiency, they noted, resulted in several clinical symptoms: retarded growth, corneal opacities, changes in skin, hair and hoofs and terminal collapse associated with hypoglycemia.

Lehrer and Wiese (1953) made a study of riboflavin deficiencies in baby pigs using a purified diet. They found that a deficiency of the vitamin is associated with alopecia, anorexia, poor growth, rough hair, dermatitis, scours, ulcerative colitis, inflammation of anal mucosa,

vomiting, light sensitivity, unsteady gait and many abnormal internal complications. Supplementing the diet with 1 to 1.5 mg. riboflavin per day for 16 days cured the external symptoms, however many internal tissue changes were not cured. The latter may have been due to insufficient riboflavin, too short a treatment or to the fact that the tissues had been permanently damaged.

As further proof that riboflavin is essential for the health and reproduction of swine an experiment by Miller _et al._ (1953) may be cited. Using a practical ration it was found that a level of 0.55 mg. of riboflavin per lb. of feed did not meet the requirement for optimum growth and was inadequate for reproduction. A level of 0.83 mg. per lb. of feed appeared to be barely adequate for the gestation period and deficient for optimum lactation. The level of 1.26 mg. per lb. of feed gave results which indicated that this is probably the practical minimum level to be recommended.

Van Poucke and Krider (1946) reported that pigs fed 1.5 mg. of riboflavin per pound of purified diet made more rapid and efficient gains than when the riboflavin level was 0.5 or 0.75 mg. per pound. Krider _et al._ (1949) working with weanling pigs in drylot and feeding a practical ration determined that 1.4 mg. of riboflavin per pound of feed was the practical minimal requirement; however no deficiency symptoms were noted, except slow growth, when the pigs

received purified diets containing 0.5 - 0.75 mg. of riboflavin per pound. A further observation was that crystalline riboflavin had the same effect as an equal amount of the vitamin from dried fermentation solubles.

Hughes *et al.* (1950) recommended a level of 0.80 mg. of riboflavin per lb. of feed while Miller and Ellis (1951) suggest that 0.83 mg. of riboflavin per lb. of feed is adequate for growing pigs kept under practical conditions in dry lot.

It has been shown that the riboflavin requirement of the pig is not constant but varies with changes in temperature (Mitchel *et al.*, 1950). Using a semi-purified diet these workers fed varying levels of riboflavin to two groups of growing pigs; the first group being maintained at a temperature of 85°F. and 50% relative humidity, the second at 42°F. and 70% relative humidity. It was found that the riboflavin requirement at the lower temperature was considerably greater than that at the higher temperature, the requirements being: 1.2 (or somewhat less) p.p.m. at 85°F. and 2.3 p.p.m. at 42°F. These values are equivalent to 0.54 and 1.04 mg. per lb. of feed and 1.4 and 4.2 mg. per 100 lb. body weight, respectively. The authors suggest that the riboflavin requirement of the pig should be expressed as per unit of air dry feed and not as per unit of body weight.

Forbes and Haines (1952) studied the riboflavin requirement of the baby pig at an environmental temperature

of 85°F. and a relative humidity of 70%. Using a 'synthetic milk diet' they found that the baby pig required between 1.5 and 2.0μ per gm. of dry feed. This level is equivalent to between 0.68 and 0.91 mg. per lb. of dry feed.

Miller et al. (1954) used a 'synthetic milk diet' to determine the riboflavin requirement of the baby pig and concluded that the optimal level was 3.0 mg. per Kg. of solids. External, gross and microscopic lesions were only present in those animals receiving less than 2.0 mg. of riboflavin per Kg. of solids. The recommended optimal level is equivalent to 1.36 mg. per lb. of dry matter in the feed.

The only work on the riboflavin requirement of pigs in Alberta is reported by Draper and McElroy (1948). Feeding a typical Western Canadian ration of oats, barley and a protein-mineral supplement along with additional vitamins A and D to growing swine it was found that additional riboflavin at the level of 1.6 gm. per ton of feed (0.8 mg. per lb.) gave no increase in growth rate or feed efficiency. It would therefore appear that the basal ration contained sufficient riboflavin to meet the requirements of these animals. It was calculated that the riboflavin content of the ration was approximately 0.8 mg. per pound.

To conclude this review of the literature on the riboflavin requirement of swine the following table is presented.

Table 1

Riboflavin Requirements of Swine

Workers	Age of pig	Type of ration	Riboflavin in mg./lb. feed	Year
Hughes	Growing	Purified	0.2 - 0.6*	1940
Van Poucke and Krider	Growing	Purified	1.5	1946
Draper and McElroy	Growing	Practical	0.8	1948
Krider *et al.*	Weanling	Practical	1.4	1949
Mitchell *et al.*	Growing	Semi-purified	0.54 - 1.04**	1950
Hughes *et al.*	Growing	Practical	0.8	1950
Miller and Ellis	Growing	Practical	0.83	1951
Forbes and Haines	Baby	Synthetic	0.68 - 0.91***	1952
Miller *et al.*	Pregnant and lactating sows	Practical	1.25	1953
Miller *et al.*	Baby	Synthetic	1.36	1954

* Originally given as 1-3 mg. per day per 100 lb. of pig from which data the figure in Table 1 was calculated.

** 0.54 mg. at 85°F. 50% relative humidity.
1.04 mg. at 42°F. 70% relative humidity.

*** At 85°F. and 70% relative humidity.

Vitamin A and riboflavin in sows' milk

Oxytocin, a hormone of the posterior pituitary gland, has been shown to cause milk ejection (Gaines, 1915 and Turner and Slaughter, 1930). Ely and Petersen (1941) applied this knowledge to milk let-down in cows and achieved complete evacuation of the mammary gland. This technique, which has allowed an improved and more complete study of the composition of milk, was first applied to swine by Braude _et al._ (1947).

Bowland _et al._ (1949a) have reviewed the literature relating to the vitamin A content of sows' milk and colostrum. Prior to this publication the most comprehensive studies of the vitamin content of sows' milk were reported by Braude _et al._ (1946, 1947).

The vitamin A content of sows' colostrum is considerably higher than that of milk (Schofield _et al._, 1942; Benham, 1943; Braude _et al._, 1947; Heidebrecht _et al._, 1948; Bowland _et al._, 1949a, 1951). That the vitamin A content of the colostrum and milk is influenced by the prepartum ration is indicated by the above workers and also by Thomas _et al._ (1947), Whiting _et al._ (1949) and Heidebrecht _et al._ (1951). Luecke _et al._ (1947) were unable to show breed differences in the vitamin A content of colostrum of swine but noted that their findings were inconclusive due to the small number of samples.

The vitamin A content of sows' colostrum and milk as

reported by some of the previously named workers are as follows:

Reported by:	Colostrum	Milk	Year
Benham	291-564μ/100 ml.	-	1943
Braude *et al.*	71.1 I.U./gm. fat	11.0 I.U./gm. fat*	1947
Thomas *et al.*	169μ/100 ml.	-	1947
Heidebrecht *et al.*	112.60μ/100 ml.	14.1μ/100 ml.**	1948
Whiting *et al.*	24μ/gm. fat	-	1949
Bowland *et al.*	136.8μ/100 ml.	46.3μ/100 ml.**	1949b

* Average for all sampling dates.

** 56th day of lactation.

There are very few reports in the literature on the riboflavin content of sows' milk and colostrum. Braude *et al.* (1946, 1947) reported an average level of 46μ/100 ml. with extremes for colostrum in 1946 of 21 and 78μ and an average value of 44.9μ/100 ml. in 1947. The average riboflavin content of sows' milk reported in the 1947 work was given as 45.7μ/100 ml., the winter level being 43.1 and the summer 48.2μ; the latter two values were not significantly different.

Davis *et al.* (1951) reported that the riboflavin content of swine colostrum was considerably greater than that of subsequent milkings, averaging 5.0 ± 2.7μ/ml. as compared with values of 2 to 3μ/ml. for milk. Luecke *et al.* (1947) reported levels of 4.00μ/ml., similar to those of Davis and her co-workers.

The apparent discrepancy between the levels determined by the English group and the two American groups will be considered in some detail during the discussion of the results of the riboflavin assays reported in this thesis.

Pearson and Darnell (1946) report that it is probable that the B-vitamin content of colostrum and milk, of a species for which these vitamins are a dietary essential, is influenced by dietary intake. This had previously been suggested by Randoin and Raffey (1943) who had shown that the riboflavin content of human breast milk is higher in summer than winter. They also noted that the riboflavin of such milk in France was higher in 1940 than in 1942 and attributed the difference to the change in diet due to rationing.

Barnhart et al. (1954) have shown that the riboflavin content of sows' milk is comparable to that reported by Davis et al. (1951). It was also observed that the ration of the sow influenced the riboflavin content of the milk. It was found that the riboflavin level of the milk of sows fed a ration composed of corn, soybean oil meal, tankage and minerals was significantly lower ($P =< 0.01\%$) than when the same ration plus a supplement of dehydrated alfalfa and meat and bone scraps was fed. The basal ration yielded an average level of 1.14μ/ml. of milk while the supplemented ration gave values of 1.53μ/ml. of milk.

Vitamin A in swine blood

An early report that vitamin A is present in blood is that of Fine (1933) who fed ox serum to young rats on an otherwise vitamin A deficient diet. The growth rate of these rats was such as to indicate the presence of vitamin A in the ox serum.

That use could be made of a measure of the vitamin A level of blood was stated by Davis and Madsen (1941) who claimed that it was possible to diagnose a deficiency of this vitamin in cattle, if the blood level was known. Hentges _et al._ (1952) showed that the first indication of a vitamin A deficiency in young pigs was a decrease in the blood plasma levels of this vitamin, to values below 15μ/100 ml., but it was not until the levels were below 5μ/100 ml. that visible symptoms of deficiency were observed. They also noted that increased cerebrospinal fluid pressures were associated with low blood plasma levels of vitamin A.

Grummer _et al._ (1946) note that the vitamin A content of the blood plasma of pigs was low at birth (0.1μ/100 ml.), reached a maximum of 0.34μ/100 ml. in the week old pig and decreased during the latter part of the suckling period. A lower level of vitamin A appeared in the blood of pigs following weaning. A paper by Dann (1933) on vitamin A in the calf offers a possible explanation to Grummer's work. It was found that the liver storage of vitamin A in the newborn calf is small compared with the amount in

the colostrum of its dam. An example was that where the liver contained 600 units of vitamin A there were 55,000 units available in the colostrum. Dann suggests that the vitamin A supplied by the colostrum and milk is far more important than that which the young animal has received by placental transfer.

The vitamin A content of the prepartum diet has been shown to affect the amount of this vitamin present in the liver and plasma of the newborn pig (Thomas *et al.*, 1946; Whiting *et al.*, 1949). The blood level of vitamin A appears to be governed by two major factors, namely, the dietary intake of this vitamin and its precursor carotene, and by the liver storage of the vitamin. However, other factors, it is claimed, do influence the plasma vitamin A levels of young calves (Thomas *et al.*, 1952, Thomas and Moore, 1952).

Lewis *et al.* (1941, 1942) reported that rats whose intake of vitamin A was less than 50 I.U. per day showed plasma A levels directly related to the dietary level. Increasing the daily vitamin A intake above 50 I.U. even to levels as high as 1000 I.U. per day had no appreciable effect upon the blood plasma content. The amount of vitamin A stored in the liver was shown to be directly related to dietary intake, while a further interesting observation was that there was no liver storage when the blood level fell below 37 I.U. per 100 ml. of plasma.

The amount of vitamin A stored in the liver bears no relation to the absolute blood level.

Braun (1945) explained the seasonal changes in blood vitamin A level of cattle as due to dietary changes. In summer when green feed is available the vitamin A level of plasma tends to rise and this it is claimed is due to the increase in dietary content of both vitamin A and carotene. Frey _et al._ (1947) found that the serum levels of vitamin A of calves fed a carotene free diet increased rapidly up to an intake of 100 I.U. of vitamin A per pound of body weight daily.

An observation on vitamin A and carotene in the blood of children shows that the serum level of these two compounds is related to the dietary intake (Merrow _et al._, 1952). Another paper dealing with the plasma vitamin A and carotene content of children is that of Szymanski and Longwell (1951). The latter workers note that human infants show a significant sex difference in both vitamin A and carotene plasma levels, the girls having the higher values. This is the only reference found which noted such a sex difference.

Milk is a food containing vitamin A and like other vitamin A containing foods, influences the amount of storage of this vitamin in suckling animals (Henry _et al._, 1949; Sobel and Rosenberg, 1950).

Haemoglobin

The earliest reference available regarding anaemia in pigs is one published by McGowan and Crichton (1923). The authors described a disease which was due to an iron deficiency and listed anaemia, thumps, oedema and heart dilation as the principal symptoms. A further paper by the same workers (McGowan and Crichton, 1924), noted that the anaemia found in suckling pigs was probably due to the low iron content of sows' milk. It was found that mixing ferric oxide with the sows' feed prevented the occurrence of anaemia, but it was suggested that the reason for this was not due to a change in the iron content of the sows' milk but rather to the accessibility of a rich store of iron for the young pigs, it having been noted that young pigs tend to root in both the sows' feed and faeces.

Elvehjem _et al._ (1927) demonstrated that raising the iron content of the rations of cows and goats did not increase the iron content of the respective milks. Due to difficulties in collecting samples of milk from the sow the effect of dietary level on the iron content of the milk could not be demonstrated but it was believed that the same fundamental principle applied.

It has been reported that cows' milk contains 0.002% and sows' milk 0.009% iron (Konig, 1920 as reported by McGowan, 1923).

In 1928 Hart _et al._ reported that copper is an essential

supplement to iron for normal haemoglobin synthesis. Hart et al. (1930) again demonstrated that the administration of soluble iron and copper salts cure anaemia; they observed that though extra iron fed to the sow appears to have a beneficial effect upon anaemic pigs this is probably only due to the iron contained in the sows' faeces in which the young pigs will root.

Until 1930 the principal method of treating anaemia in young pigs was to give soluble iron salts. However, in that year Schofield (1930) reported that reduced iron was just as effective and more easy to administer.

Hamilton et al. (1933) define clinical anaemia in pigs as the condition that results when the blood haemoglobin concentration reaches a level of 3.5 gm. or less per 100 ml. of blood. They also reported that the average haemoglobin level of the newborn pig is 11.5 gm. percent.

According to Moe et al. (1935) pigs receiving various iron supplements made no better growth than pigs receiving no supplements providing the latter did not develop clinical anaemia. However, taking all groups into consideration, the groups receiving iron were significantly heavier at weaning than the groups receiving no iron. Presumably the latter groups would include certain animals suffering from clinical anaemia.

Swales et al. (1942) note that many litters born in Canada are, of necessity, farrowed indoors without access

to soil or vegetation. A drop in the haemoglobin level of such pigs occurs in the first 10 days of life from a level approximating that of the adult to one bordering on clinical anaemia.

The rate of growth of young pigs tends to decrease between the first and fourth weeks of life. The flattening of the growth curve was modified by Swales *et al.* (1942) when pigs were given iron. It would seem probable therefore that a lowered concentration of blood haemoglobin, though not reaching the point of clinical anaemia has a direct effect upon gain in weight. It is suggested that iron should be given within two days after birth and then when the pigs are ten days old, as advised by Schofield (1930). Experiments conducted in Alberta by Draper and McElroy (1949) resulted in the observation that suckling pigs should be treated with reduced iron at weekly intervals, the first dose to be administered on or before the third day after birth.

Byers *et al.* (1952) working with cattle found no difference in the haemoglobin levels of different sexes but did notice a significant breed difference. A sex difference in dairy calves has been noted by Thomas *et al.* (1954). From birth to seventy-five days of age females had a significantly higher haemoglobin level than male calves. Soon after this age had been reached the males achieved a haemoglobin level which was greater than that of the females.

CREEP FEED RATIONS

Objective

The objective of this experiment was to study the addition of supplemental riboflavin and/or feed flavour to a creep feed ration for suckling pigs.

Experimental

The farrowings of the purebred Yorkshire sows at the University Farm during 1954 occurred in four definite periods; these were January, February-March, July and September. Prior to farrowing, each sow had been allotted to one of four groups for the purpose of conducting a creep feed experiment with the resulting litters. The rations received by these sows during both pregnancy and lactation are listed in Table 2. It should be noted that the sows farrowing in the summer had been on pasture until a few days prior to parturition hence the absence of both alfalfa and vitamin A and D supplement in their rations.

Sows were weighed prior to farrowing, eight hours after farrowing, and subsequently at weekly intervals until weaning. Litter weights of the young pigs born in January and February-March were obtained at birth, one, two, four and six weeks of age, and at weaning at fifty-four* days of

---

* January litters were weaned at 54 rather than 56 days of age because of a shortage of farrowing space. Future litters were weaned at this same age to prevent variability in weaning ages.

Table 2

Sow Rations for Creep Feed Experiment (lb.)

	Pregnancy[*]		Lactation
	Spring[**]	Summer	
Barley	600	600	500
Oats	1200	1200	1200
Wheat			200
Alfalfa meal	200		
Concentrate (35%)	200	200	200
Linseed oil meal			100
Wheat bran	100	100	100
Feeding oil[***]			3
Iodised salt	12	12	12
Ground limestone	12	12	12
TOTAL	2324	2124	2327

[*] Spring relates to farrowings in January and February-March. Summer relates to farrowings in July and September.

[**] Also received an average of 1000 I.U. of vitamin A and 150 I.U. of vitamin D_3, administered as a feeding oil spread on top of the feed, once per day.

[***] Feeding oil contained 1000 I.U. vitamin A and 150 I.U. vitamin D_3 per ml.

age. Litters born during the summer were weighed at weekly intervals following birth.

When the young pigs were two weeks of age they were offered solid feed behind a creep in the corner of the pen. The creep feed ration offered was that appropriate to the group to which the litter had been allotted prior to birth; a weighed amount being placed in a self feeder which was periodically replenished. At weaning the amount of feed left in each creep was carefully weighed and the amount consumed by the litter, while suckling the sow, was computed.

The four creep feed rations were as listed below:

1. Basal

Wheat (coarsely ground)	55 lb.
Oat groats (coarsely ground)	22 lb.
Meat scraps	4 lb.
Soybean oil meal	7 lb.
Sugar (granulated white sucrose)	10 lb.
Vitamin B_{12} - Aureomycin supplement*	1 lb.
Iodized salt	0.5 lb.
Ground limestone	0.5 lb.

Dry "A" and Dry "D" to supply 100,000 I.U. of vitamin A and 20,000 I.U. of vitamin D_2 per 100 lb.

2. Basal ration + 2 gm. riboflavin/ton.

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* Lederle Aurofac Supplement containing 1.8 mg. of vitamin B_{12} and 1.8 gm. of aureomycin hydrochloride (chlortetracycline) per pound.

3. Basal ration + 1 lb. Feed Flavor*/ton.
4. Basal ration + 2 gm. riboflavin + 1 lb. Feed Flavor/ton.

The creep feed was mixed in 500 lb. lots unless it was obvious that less was required. Each mix was sampled and nitrogen and crude fibre content was determined in the laboratory. The riboflavin content of the ration was calculated from the values given in the Appendix, table 5 of Morrison's Feeds and Feeding, 21st Ed.

Analysis of the various mixes of the creep feed ration indicated that the protein content was approximately 16% the range being from 15.7% to 16.7%. Crude fibre content of the creep feed rations never exceeded 3% the range being 2.5 to 2.9 per cent. The calculated riboflavin content of the basal creep feed ration (Morrison, 1948) was approximately 1.2 gm. per ton of feed.

Results

A summary of the data obtained from the experiment previously described is given in tables 3, 4 and 5. The growth rate of litters by farrowing periods is expressed graphically on page 28, while the growth rates of the different ration groups, irrespective of date of birth, are indicated in a similar manner on page 29.

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* Anifeed Dribase Pig Feed Flavor containing extractives of anise, cinnamon, clove, birch, orris, butyric acid, vanillin, soya meal and corn sugar.

Data such as those presented in tables 3, 4 and 5 are extremely difficult to analyze statistically for a great many factors have a potential influence on the weaning weight of a pig or a litter. A few of these variables are: birth weight, amount of milk received (this is probably influenced by both feed consumption and loss in weight of sow), number and sex of pigs, creep feed consumption and several other minor factors. In spite of such difficulties a certain amount of statistical analysis has been conducted and is reported in the following pages.

Prior to conducting the analysis it was necessary to eliminate one sow and litter from ration two (basal + 2 gm. riboflavin/ton) in order that there would be twelve sows and litters in each ration group. The method chosen for selecting the particular sow and litter to be eliminated was as follows: it was noted that in the February-March farrowing group there were four sows and litters on ration 2 while only three on rations 1 and 3 and two on ration 4. It was decided that a sow and litter from this particular farrowing should be eliminated, further selection as to the individual being made by drawing a number from a hat.

Although the sows were selected at random it was decided to carry out a preliminary analysis of variance of the total birth weight of the litters and also of the average birth weight of the pigs, between ration groups, to

Table 3

Average Litter Weights (lbs.)

	No. of litters	Birth		28 Days		54 Days	
		Mean	Range	Mean	Range	Mean	Range
DATE OF FARROWING							
January	10	25.3	16.0-30.1	127	94-171	232	178-304
Feb.-March	12	23.1	15.1-34.2	114	68-142	209	122-277
July	12	28.9	15.0-38.0	154	109-219	353	216-463
September	15	28.0	17.7-39.0	136	86-171	320	180-419
RATION							
Basal	12	25.4	15.1-33.0	128	68-171	274	122-419
+ riboflavin	13	24.5	15.0-32.0	133	86-167	266	180-364
+ flavor	12	28.6	21.1-39.0	145	123-177	307	178-397
+ riboflavin and flavor	12	27.0	16.0-38.0	162	92-219	287	179-463
Total Mean	49	26.5	15.0-39.0	135	68-219	283	122-463

Table 4*

Factors Having a Potential Influence on the Data of Table 3

Date of farrowing	Change in wt. of sow**		Feed consumed by sow		Creep feed consumed per litter	
	Mean	Range	Mean	Range	Mean	Range
January	-90	-(22-175)	735	572-818	47	14-107
Feb.-March	-68	-(23-152)	766	624-910	79	15-205
July	-69	-(27-101)	891	691-693	204	15-315
September	-76	-(23-147)	859	821-890	200	27-340
RATION						
1	-72	-(25-122)	811	624-951	114	15-265
2	-83	-(23-175)	821	647-963	119	20-340
3	-81	-(22-175)	836	710-930	150	14-267
4	-64	-(22-104)	809	572-917	159	14-315
Total mean	-75	-(22-175)	819	572-963	135	14-340

---

* All data expressed as pounds.

** Weight just prior to farrowing minus weight at time of weaning.

Table 5

Average Pig Weights and Creep Feed Consumption (lb.)

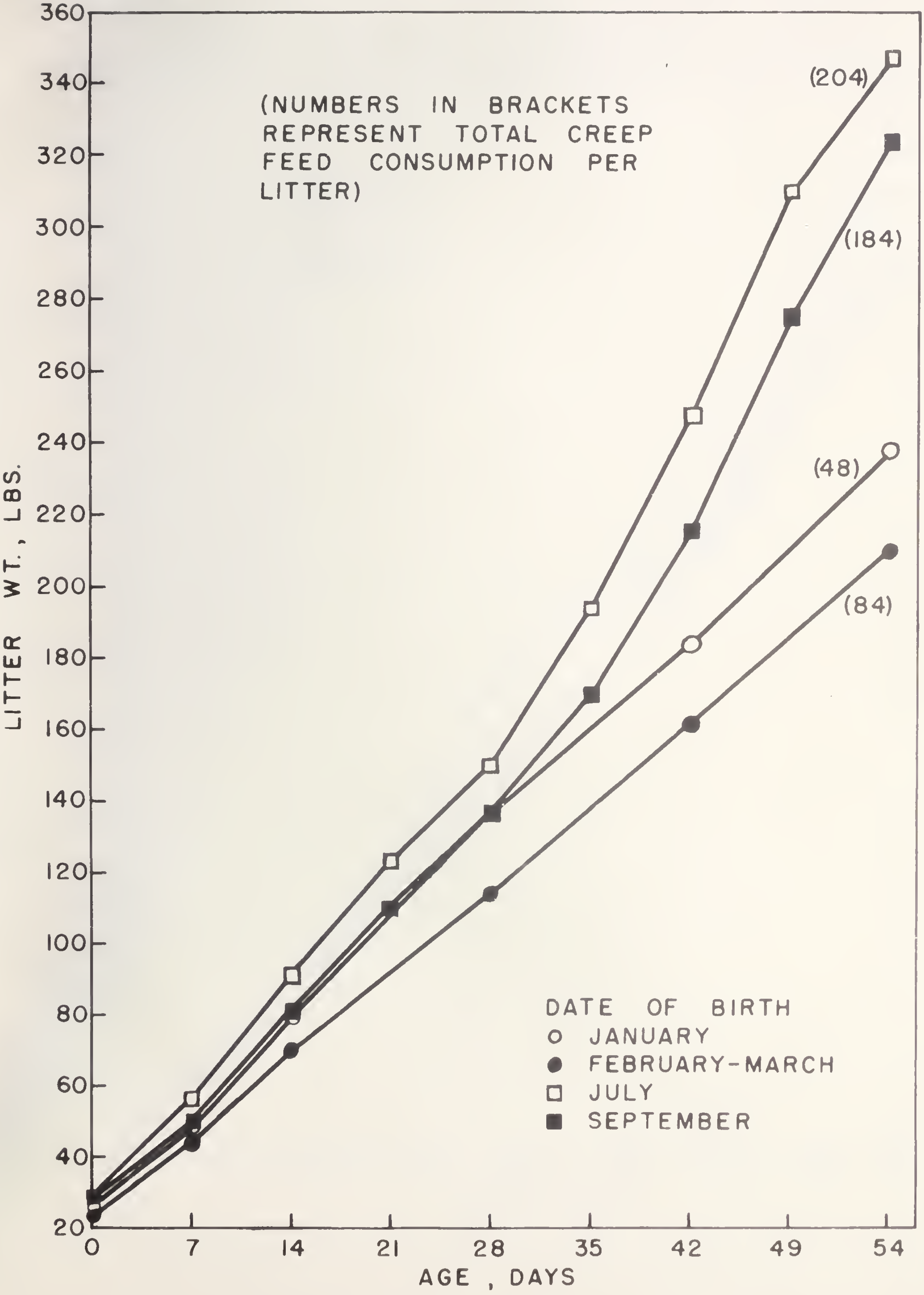
	Age of pigs			Creep feed consumed per pig
	Birth	28 days	54 days	
DATE OF FARROWING				
January	2.61	14.34	25.22	5.12
Feb.-March	2.86	13.42	24.85	9.33
July	2.78	16.40	37.15	21.89
September	2.58	14.17	33.60	18.03
RATION				
Basal	2.58	14.41	30.42	12.62
+ riboflavin	2.65	15.59	32.20	14.74
+ flavor	2.58	13.73	29.22	14.31
+ riboflavin and flavor	2.75	14.81	32.18	17.80
Total mean	2.64*	14.60	30.82**	14.72

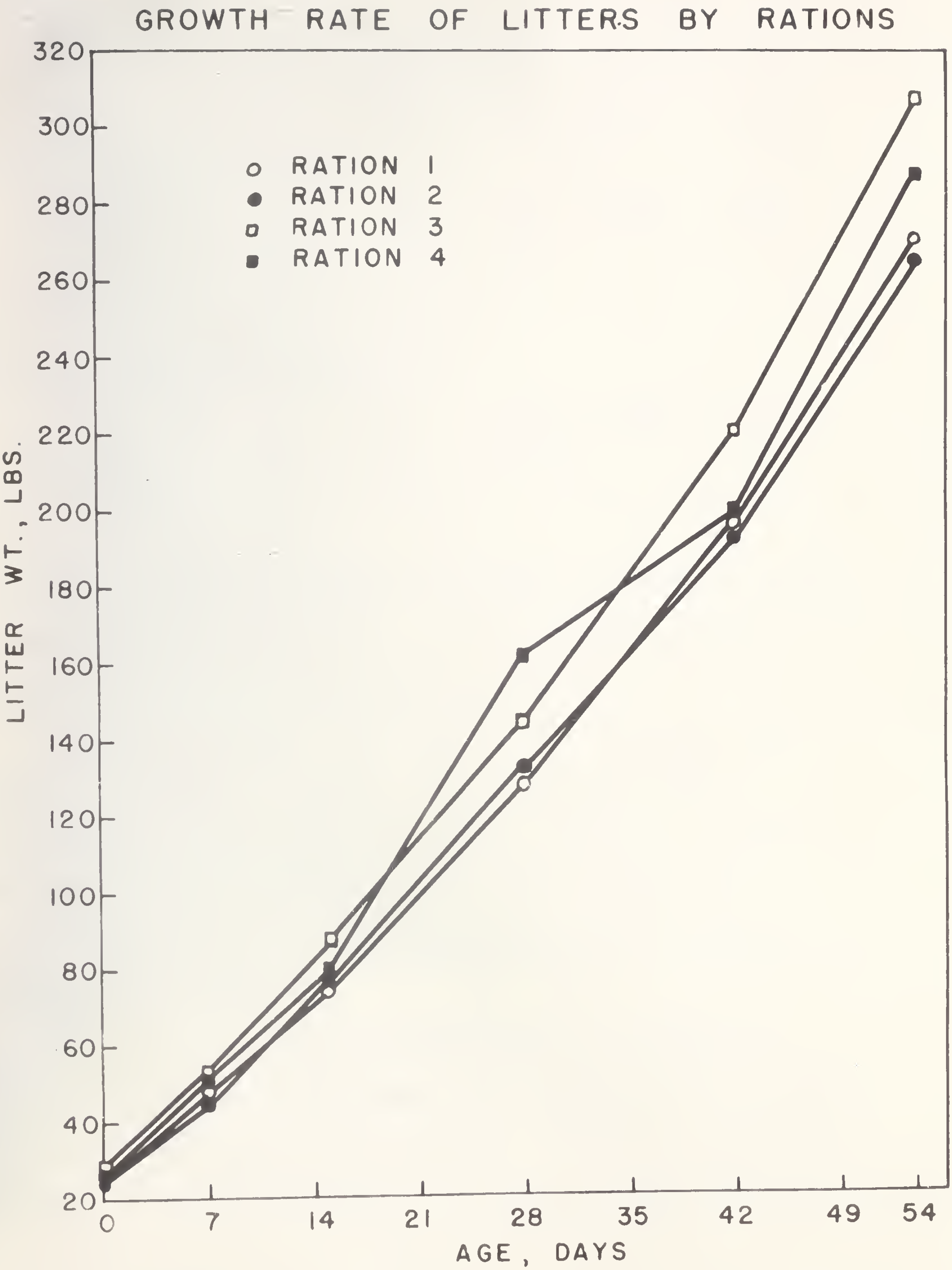
* 492 pigs born averaging 10.04 per litter.

** 450 pigs weaned averaging 9.18 per litter.

Figure 1

GROWTH RATE OF LITTERS BY FARROWINGS




GROWTH RATE OF LITTERS BY RATIONS
○ RATION 1
● RATION 2
◻ RATION 3
■ RATION 4
LITTER WT., LBS.
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
7
14
21
28
35
42
49
54
AGE, DAYS

determine whether randomization had been successful in regard to these measures.

Analysis of Variance

	D.F.	S.S.	M.S.	F.	5%
1. Birth weight of litters					
Rations	3	145	48	1.41	2.82
Error	44	1,499	34		
Total	47	1,644			
2. Birth weight of average pigs					
Rations	3	0.17	0.057	0.39	2.82
Error	44	6.42	0.146		
Total	47	6.59			

This analysis would indicate that in relation to the birth weights of litters and average pigs the random selection of the sows had been successful.

A further analysis was conducted to determine the effect of rations on weaning weight and creep feed consumption with any seasonal effects removed. As with the data relating to birth weight this analysis was first conducted on a litter weight basis and secondly on an average pig basis.

The reason for analysing thesedata in relation to both litter weights and average pig weights was to determine whether the variation in the number of pigs per litter had any major significant effect on weaning weights and creep

Analysis of Variance

	D.F.	S.S.	M.S.	F.	5%	1%
1. Weaning weight of litters						
Rations	3	10,051	3,350	1.04	2.90	4.46
Seasons	3	167,519	55,840	17.31	2.90	4.46
R X S	9	43,078	4,786	1.48		
Error	32	103,224	3,226			
Total	47	323,872				
2. Creep feed consumption of litters						
Rations	3	15,414	5,138	0.914	2.90	4.46
Seasons	3	206,758	68,919	12.26	2.90	4.46
R X S	9	44,600	4,956	0.88		
Error	32	179,956	5,624			
Total	47	446,728				
3. Weaning weight of average pigs						
Rations	3	66.56	22.19	1.13	2.90	4.46
Seasons	3	1439.72	479.91	24.47	2.90	4.46
R X S	9	40.12	4.46	0.23		
Error	32	627.66	19.16			
Total	47	2174.06				
4. Creep feed consumption of average pigs						
Rations	3	201.49	67.16	1.14	2.90	4.46
Seasons	3	1894.13	631.38	10.76	2.90	4.46
R X S	9	370.90	41.21	0.70		
Error	32	1877.08	58.66			
Total	47	4342.60				

feed consumption. Examination of the analysis indicates that there was no difference between the results obtained irrespective of whether litter weights or average pig weights were used in the analysis.

The analysis indicates that there was no significant difference between the weaning weights or creep feed consumption of the different ration groups. There was, however, a highly significant seasonal difference in both weaning weights and creep feed consumption.

A total gross correlation between the weaning weight and creep feed consumption of the litters under test yielded an r value of 0.853 at 46 D.F. which is significant at the 1% level. This would indicate that approximately 73% of the variation in weaning weight was associated with the amount of creep feed consumed. Due to the fact that a significant seasonal variation in both weaning weight and creep feed consumption had been observed doubts could be expressed as to the validity of the above data and a similar correlation within periods was measured. The latter measurement removed seasonal effects from the correlation and yielded an r value of 0.733 at 40 D.F. which is also significant at the 1% level. This new value for r indicates that when seasonal influences are removed only 60% (approximately) of the variation in weaning weight is associated with creep feed consumption.

Since total litter weight and creep feed consumption could be influenced by the number of pigs in the litters, the correlation between average pig weight at weaning

and average creep feed consumption per pig within seasons was measured. The value obtained for r was 0.465 which is again significant at the 1% level. This r value (40 D.F.) indicates that only 22% (approximately) of the variation in average pig weaning weights within periods is associated with creep feed consumption. This latter value is probably the most accurate measure it is possible to make from the available data, for in its calculation the variations due to both seasons and number of pigs in a litter are eliminated.

Discussion

The explanations and interpretations of the statistical analysis presented in the foregoing section are sufficiently comprehensive as to remove the necessity of a detailed discussion.

The analyses of variance would indicate that there was no significant difference between the four ration groups respecting birth weight, weaning weight or creep feed consumption. It would therefore follow that there was no preference for any one ration, nor had any ration a superior nutritive value. This would indicate that, under the particular conditions of this trial, Anifeed Dribase Pig Feed Flavor did not increase the palatability of the creep feed ration above a level which could be easily explained by chance fluctuations. These results bear a similarity to those

published at Minnesota by Hanson *et al.* (1954), where an anise-molasses mixture did not increase the palatability of a creep feed ration. It is still quite possible that feed flavours may be of value in creep feed rations but this trial would indicate that the particular flavour used had no demonstrable beneficial effect.

That the nutritive value of the basal creep feed ration did not appear to be increased by the addition of 2 gms. of riboflavin per ton would indicate that the content of the basal ration (calculated 1.2 gm./ton) was sufficient for the needs of the suckling pigs. It is difficult to compare this value with those previously given in table 1 as the suckling pigs would also receive an unmeasured quantity of riboflavin from the sows' milk. However it is felt that the value of 1.2 gm./ton or 0.6 mgm./lb. of feed is not very different from the requirements of the pig as already listed. It seems that the riboflavin content of the basal creep feed ration did not limit the growth rate of suckling pigs.

Summary

1. A correlation calculated between the average pig weaning weight and creep feed consumption indicated that only 22% (approximately) of the weaning weight variation was associated with creep feed consumption.

2. Addition of 2 gm. of riboflavin per ton of creep feed did not appear to affect the growth rate of the suckling pigs.
3. Anifeed Dribase Pig Feed Flavor did not appear to increase the palatability of the basal creep feed ration.

THE VITAMIN A AND RIBOFLAVIN CONTENT OF SOWS' MILK

Objective

The following experiment was initiated in order to determine whether there were any significant seasonal changes in the vitamin A and riboflavin content of sows' milk; taking into account the fact that sows farrowing in January and February-March received different prepartum rations to those farrowing in July and September.

Experimental

Prior to farrowing one sow from each ration group for each farrowing period, from the creep feed study previously discussed, was selected for milk study. The milking technique was as follows. The sow to be milked was held by a rope noose placed around the snout. One ml. of Pitocin*, was then injected intravenously into the ear. Two milkers then proceeded to draw milk into amber coloured glass bottles, the aim being to completely milk two teats though towards the end of the lactation period it was sometimes necessary to draw milk from several teats to obtain a sufficiently large sample. The two samples of milk were then pooled and the container stoppered and placed in a wooden box for transfer to the laboratory.

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* Parke Davis Pitocin containing 10 I.U. of oxytocin per ml. ampule.

On arrival at the laboratory the milk was placed in a refrigerator until assayed. In the majority of cases the assays were set up within a few hours of the collection of the samples and in no instance was the raw milk stored for a period greater than three days.

The methods of analysis used for the assay of both these vitamins are given in the Appendix. However it is necessary to outline the method of extraction for riboflavin as the assay values obtained are somewhat different from those of other recent workers in this field, for reasons which will be considered in the Discussion.

A 25 ml. aliquot of milk was placed in a 125 ml. Erlenmeyer flask containing 12 ml. of 0.2 $N.H_2SO_4$. The flask was plugged with cotton wool and then autoclaved at 15 lb. pressure for 15 minutes. On cooling the flasks were placed in the dark in a deepfreeze until the assay could be completed. This method of extraction is identical with that given in the laboratory manual for Biochemistry 125, University of Wisconsin, and is essentially similar to the extraction methods of Hodson and Norris (1939), Slater and Morell (1946), Snell (1950), Stiller (1951), and the Association of Vitamin Chemists (1951).

Results

The sows used in this experiment farrowed at four different periods of the year so that the rations fed

during pregnancy differed though they all received the same lactation ration. The prepartum ration of the sows farrowing in January and February-March contained alfalfa meal and was fortified with vitamins A and D. The sows farrowing in July and September received neither alfalfa nor the vitamin supplements but had access to pasture until a few days prior to farrowing. It was therefore decided to tabulate all data according to farrowing dates and stage of lactation.

It will be noted that sows of the first two farrowing periods were not milked on the 21st, 35th and 42nd day of lactation. There were, as stated previously, four sows in each farrowing group.

Table 6

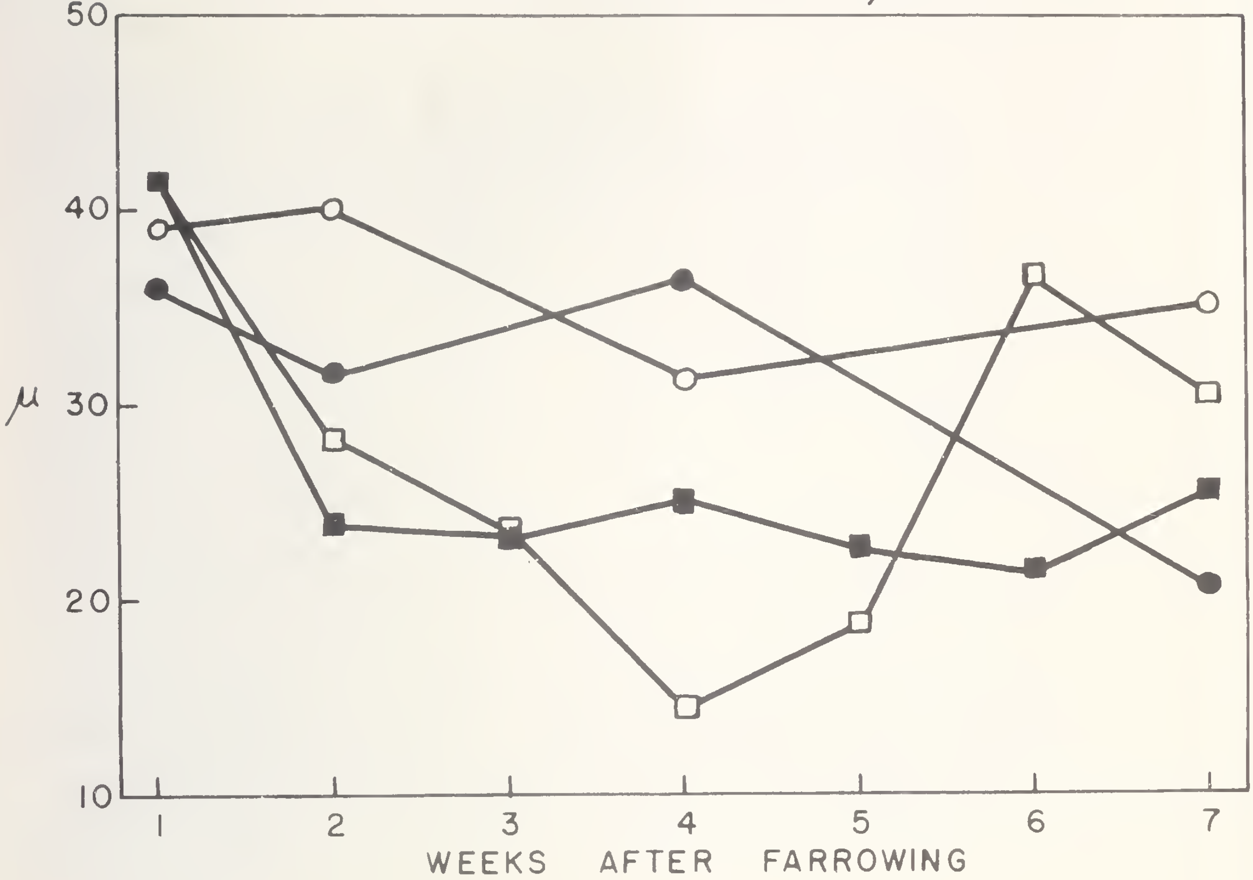
Vitamin A Content of Sows' Milk (μ/100 ml.)*

Date of Farrowing	Day of Lactation 7th	14th	21st	28th	35th	42nd	49th
January	39.2	40.1		31.3			35.1
Feb.-March	36.0	31.5		36.4			20.6
July	41.5	28.3	23.6	14.5	18.8	36.6	30.5
September	41.7	23.9	23.2	26.1	22.7	21.5	25.6
Average	39.6	31.0		27.1			28.0

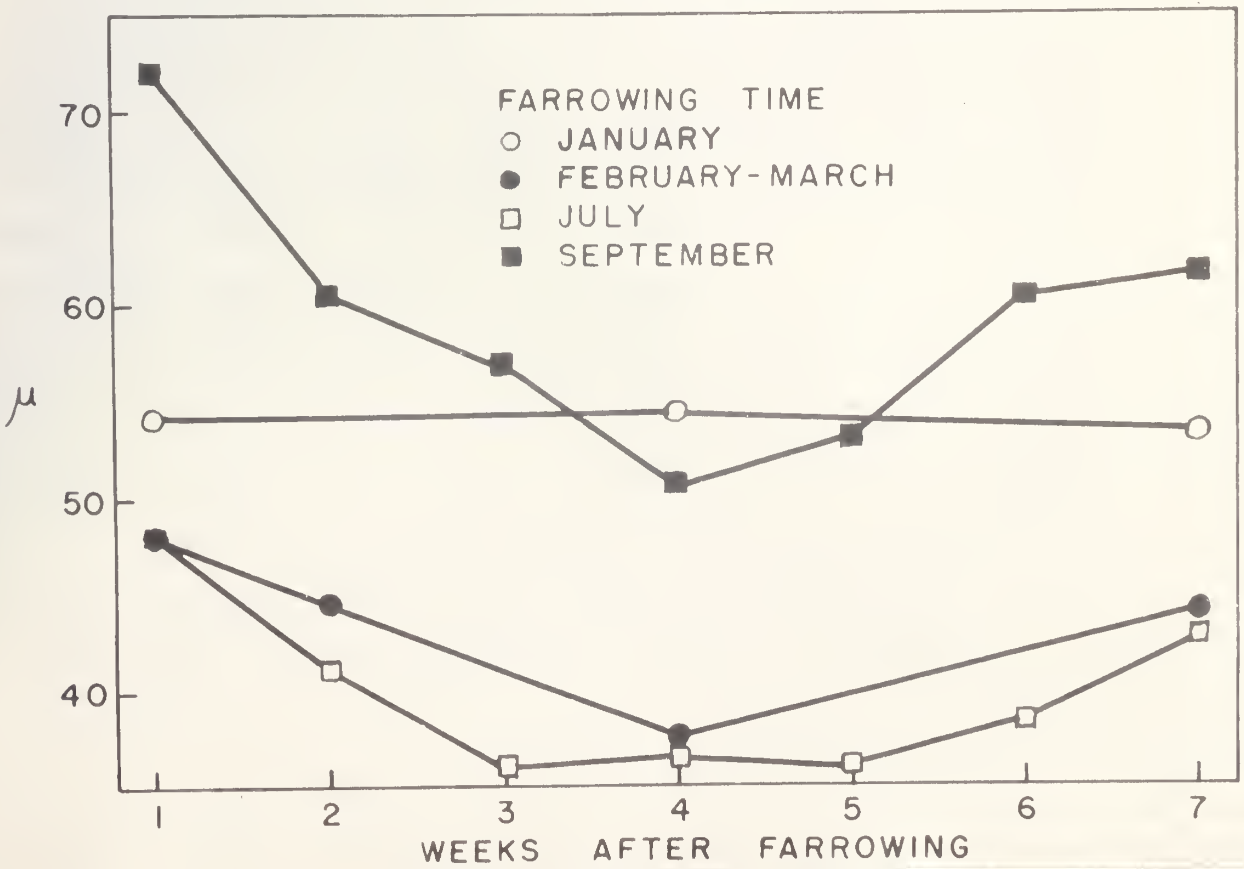
* These data are shown as a series of graphs on page 38.

An analysis of variance conducted on the data for the 7th, 14th, 28th and 49th day of lactation indicated no

Figure 3



VITAMIN A IN SOWS MILK (μ/100 ML.)



RIBOFLAVIN IN SOWS MILK (μ/100 ML.)

significant difference in the vitamin A content of sows' milk between farrowing periods or stage of lactation.

Analysis of Variance for Vitamin A in Sows' Milk

	D.F.	S.S.	M.S.	F.	5%
Farrowing	3	589	196	1.11	3.86
Lactation	3	1574	525	2.98	3.86
F x L	9	1582	176	2.47	2.08
Error	48	3410	71		
Total	63	7155			

As the F value for the interaction (F x L) was significant at the 5% level the interaction M.S. was used in the calculation of the F values for both farrowing and lactation.

Table 7

The Riboflavin* Content of Sows' Milk (μ/100 ml.)**

Date of Farrowing	Day of Lactation 7th	14th	21st	28th	35th	42nd	49th
January	54.2			54.4			54.0
Feb.-March	48.0	44.5		37.5			44.2
July	48.0	41.2	36.0	36.4	36.0	38.3	42.8
September	72.2	60.6	56.9	50.7	53.1	60.5	61.6
Average	55.6			45.0			50.7

* Later referred to as 'acid liberated riboflavin.'

** These data are also shown as a series of graphs on page 38.

An analysis of variance conducted on the data available for the 7th, 28th and 49th day of lactation yielded the following results.

Analysis of Variance for Riboflavin in Sows' Milk

	D.F.	S.S.	M.S.	F.	5%	1%
Farrowing	3	3,025	1,008	10.39	2.86	4.38
Lactation	2	947	474	4.89	3.26	5.25
F x L	6	475	79	0.81	2.36	3.35
Error	36	3,480	97			
Total	47	7,927				

These results indicate a highly significant variation between farrowings while there is also a significant variation between stages of lactation.

The mean values for all measurements made on milk obtained from the 7th, 28th and 49th day of lactation were as follows:

Farrow 1 (January)	54.2μ/100 ml.
Farrow 2 (Feb.-March)	43.2μ/100 ml.
Farrow 3 (July)	42.4μ/100 ml.
Farrow 4 (September)	61.5μ/100 ml.

An L.S.D. value for farrowings was calculated and found to be 10.95 at the 1% and 8.16 at the 5% level of "t". From the results of the analysis it would appear that the milk collected from sows farrowing in January and September had

a significantly higher ($P = < 0.01$) riboflavin content than milk collected from sows farrowing in February-March and July.

Discussion

Vitamin A.

The failure of the vitamin A content of the sows' milk to show significant differences either between farrowings or between different stages of lactation is rather interesting. It has been reported that the vitamin A level of sows' milk and colostrum is influenced by the vitamin A and carotene content of the prepartum diet (Schofield *et al.*, 1942; Benham, 1943; Braude *et al.*, 1947; Thomas *et al.*, 1947; Heidebrecht *et al.*, 1948, 1951; Whiting *et al.*, 1949; Bowland *et al.*, 1949a, 1951). The sows of the first two farrowing periods received a pregnancy ration containing both alfalfa and mixed feeding oil while those of the latter two farrowings received neither of these supplements though they did have access to pasture until a few days prior to parturition. It would therefore appear that all sows received sufficient vitamin or pro-vitamin A in their pregnancy ration, to give the maximum amount of vitamin A in their milk. It would seem possible that the inclusion of even larger doses of vitamin A in the ration would not have affected the milk content of this vitamin as the sows were already receiving an amount in excess of the

minimum optimal requirement. The small number of sows used in this experiment, four groups of four, does not allow the above postulation to be anything more than a hypothesis. The results obtained averaging between 20.6 and 35.1 μ/100 ml. of milk on the 56th day of lactation were in the range of those reported by previous workers. As shown on page 10 these values are: 14.10μ/100 ml. milk (Heidebrecht *et al.*, 1948) and 46.3μ/100 ml. milk (Bowland *et al.*, 1949b).

Riboflavin.

Before entering into a discussion of the results obtained from this experiment it is necessary to explain why the riboflavin values obtained by Braude *et al.* (1947) and those reported in this thesis are considerably lower than those reported by Luecke *et al.* (1947) and Davis *et al.* (1951).

Unfortunately, the report of Davis *et al.* (1951) did not come to hand until after the riboflavin assays of the samples of sows' milk, collected as previously described, had been completed. Using the method reported in the Appendix, values comparable to those obtained by Braude *et al.* (1947) were obtained while a series of recoveries gave values in excess of 98 percent. It was therefore concluded that the method of assay was acceptable.

The method used by Braude *et al.* (1946, 1947) for assaying the riboflavin content of sows' milk was that of Henry *et al.* (1939) which had been proven acceptable for

cows' milk. A certain amount of surprise resulted from the low riboflavin levels obtained by Braude *et al.* but the observed fact that the vitamin C in sows' milk and colostrum was only slightly affected by exposure to light supported the low analytical values for riboflavin which is known to act as a catalyst in the photochemical destruction of vitamin C (Kon, 1938). Davis *et al.* (1951) assayed riboflavin in sows' milk microbiologically by the method of Snell and Strong (1939) with minor modifications in the composition of the basal medium. The samples were not submitted to either acidic or enzymatic hydrolysis.
In an attempt to resolve the obvious conflicts in results, collaboration between Davis *et al.* and Braude *et al.* was undertaken. The results of these cooperative studies were presented by Davis *et al.* (1950).

The outcome of the communications between Davis *et al.* and Braude *et al.* are reported below. Using a pooled sample of freeze dried sows' milk reconstituted to 15 litres Braude *et al.* assayed aliquots for riboflavin using several different procedures and obtained the following values.

Emmerie's (1938) chemical extraction	0.4μ/ml.
Microbiological similar to that used by Davis *et al.* (1951)	1.5μ/ml.
Microbiological similar to that of Kornberg, Langdon and Cheldelin (1948) using *Leuconostoc mesenteroides*	1.2μ/ml.
Chick test with 14 birds using the method of Coates *et al.* (1948)	1.5μ/ml.
Rat tests using the method of Henry *et al.* (1940)	1.8μ/ml.

The conclusion was drawn (Davis *et al.*, 1950) that riboflavin is present in sows' milk at least partly in a form different from that in cows' milk. It was also decided that the riboflavin content of sows' milk is much higher than originally reported by Braude *et al.* (1946, 1947).

The method of extraction of riboflavin from cows' milk reported by Henry *et al.* (1939) and applied by Braude *et al.* to sows' milk was basically a methanol extraction. The sample of milk along with 95% methyl alcohol and glacial acetic acid was boiled under reflux in an atmosphere of nitrogen and then centrifuged. The solids were then extracted a second time and washed with methanol. All extracts were stored overnight in a refrigerator during which time the lactose crystallized out and was removed by centrifuging. Removal of pigments by the conventional use of $KMnO_4$ and subsequent destruction of excess $KMnO_4$ with H_2O_2 was standard practice. Evaporation of the extract in vacuo and subsequent measurement of the riboflavin fluorimetrically under ultra violet light completed the determination.

Other methods of extraction of riboflavin from samples of biological origin are given below.

Whitnah *et al.*, 1937	15 ml. 10% trichloroacetic acid to 10 ml. of milk.

Weisberg and Levin, 1937	Refluxing with methanol and acetic acid in an atmosphere of CO_2. Precipitation of flocculent materials in extract achieved by the use of acetone.
Hodson and Norris, 1939	Refluxing with 0.25 N H_2SO_4 for one hour.
Jones and Christianson, 1941	Heating in a steam bath with dilute phosphoric acid for 1 hour.
Conner and Straub, 1941	Extraction for one hour with 0.04 N H_2SO_4 in a boiling water bath followed by a clarase digestion at 45°C. for 2 hours.
Slater and Morell, 1946	Recommend autoclaving the sample with 0.25 N H_2SO_4 for 30 minutes at a pressure of 15 lbs./sq. in.
Snell, 1950	Heating sample with 0.1 N HCl is an extraction method which gives consistent values when followed by microbiological assay.
Stiller, 1951	Autoclave with HCl for 30 minutes.
Association of Vitamin Chemists	Autoclave with 0.1 N HCl for 30 minutes. This extraction followed by the fluorimetric determination as described in the Appendix is claimed to give values comparable to microbiological assays for many biological products including milk, fresh, whole or skimmed; milk, evaporated or condensed; cream, sweet or sour and buttermilk.
Laboratory Manual, 1946 Biochemistry 125, University of Wisconsin	Autoclave 25 ml. of milk with 12 ml. of 0.2 N H_2SO_4 for 15 minutes at 15 lbs. pressure.
Davis _et al._, 1951	It is pointed out by these authors that a modification of the extraction method of Conner and Straub (1941), in which a digestion with clarase and takadiastase is included, yielded an extract for fluorometric determinations which give values comparable to those obtained by means of microbiological assay.

The observation that all the riboflavin in sows' milk is not extracted by methanol, or by acid hydrolysis, as it is from cows' milk, makes a paper by Roderuck et al. (1945) of considerable interest. These workers measured the riboflavin content of human milk by two different methods. The first method was a modification of that used by Conner and Straub (1941), the extraction being achieved by autoclaving the milk with 0.1 N H_2SO_4. The second method was similar to the first though an enzymatic hydrolysis, using clarase (diastase of malt highly concentrated), was included, following the acid hydrolysis. The values obtained by the second method were considerably higher than those obtained by the former method of extraction. These workers termed the riboflavin freed by the acid hydrolysis the 'free riboflavin' while that released by the combination of acid and enzymatic hydrolysis was designated the 'total riboflavin'; the latter consisted of the 'free riboflavin' and so called 'combined riboflavin'. The total riboflavin values were found to be approximately 20% lower than check values obtained microbiologically but the microbiological method seemed less reliable since determinations could not be duplicated from day to day. In a series of determinations on one sample, spread over a period of four weeks, the microbiological method varied 27% while the chemical method varied only by 5 percent. Similar difficulties associated with the microbiological

determination of riboflavin have been reported by Andrews (1943). The results obtained by Roderuck *et al.* indicate that the 'free riboflavin' expressed as a percentage of the total riboflavin increases as lactation proceeds, the range being from 43 to 86 percent.

If we accept the concepts of free and combined riboflavin as given above, the data reported by Braude *et al.* (1946, 1947) and the data reported in this thesis assume a new value. However when discussing free riboflavin in the above sense we must not confuse it with the free riboflavin referred to by Bessey *et al.* (1949). The latter workers determined that riboflavin was present in the animal body in three forms namely, free riboflavin, flavin adenine dinucleotide (F.A.D.) and flavin mononucleotide (F.M.N.). The quantity of free riboflavin in animal tissue was found to be very small. To save confusion between these two sets of terminology a third is suggested. For the remainder of this paper, the 'free riboflavin' reported by Roderuck *et al.* will be referred to as 'acid liberated riboflavin'. The term 'combined riboflavin' may be retained as it is self explanatory.

It would appear, by definition, that the values for the riboflavin content of sows' milk reported in this thesis are really measures of the 'acid liberated riboflavin'. The statistical analysis of the data obtained has been reported earlier. The 'acid liberated riboflavin' content

of sows' milk varied significantly with the time of year at which the sows were farrowed. The reason for this could be the different prepartum diets, for the ration of the sow has been shown to influence the riboflavin content of her milk (Pearson and Darnell, 1946; Barnhart *et al.*, 1954). However it is believed that all sows received riboflavin well in excess of their minimum requirements during the prepartum period while the lactation ration was constant for the duration of the experiment. Emmerie (1936) has suggested that human subjects may become saturated with flavins and that if riboflavin in excess of normal requirements is fed in the form of riboflavin the saturation point will be reached and then all in excess of the normal requirement will be excreted in the urine. If this applies to sows then it would appear that some factor other than ration was exerting an influence on the 'acid liberated riboflavin' level of the milk. It is of course possible that excess riboflavin might be excreted in the milk but no evidence was found to support this hypothesis.

An examination of the graphs on page 38 would indicate that the 'acid liberated riboflavin' level of the milk tends to fall from the first week of lactation until the fourth after which it tends to increase again. Several workers have reported that the lactation peak is reached about three weeks after farrowing though naturally this peak will vary from sow to sow (Henry and Woll, 1897;

Hughes and Hart, 1935; Smith, 1937; Davidson, 1949; Carroll and Krider, 1950; Bowland and McElroy, 1952; Hammond, 1952). If for the particular sows under test the mean time of peak milk yield was approximately four weeks after farrowing, then it is possible to put forward the hypothesis that the excretion of 'acid liberated riboflavin' via the milk is relatively constant and that the level per 100 ml. varies according to the amount of milk being produced. The greatest objection to this hypothesis is that no reason can be given for sows farrowing in February-March and July to produce more milk than sows farrowing in September or January.

An interesting problem arises from the work of Roderuck et al. (1945). If all the riboflavin of human milk is not liberated by acid hydrolysis what are the chances of the suckling child making use of the total riboflavin of the milk? A similar problem exists in the case of the suckling pig. It is true that the total riboflavin may be liberated by enzymatic hydrolysis following an acid hydrolysis but whether sufficient hydrolysis can take place in the animal's digestive tract is doubtful.

The amino acid composition of a protein, based upon acid hydrolysis does not necessarily indicate the extent of liberation of each amino acid from the protein by enzymatic digestion (Riesen et al., 1947). These workers also indicate that a microbiological assay of the amino acids liberated by enzymatic hydrolysis gives a superior

measure of the nutritive value of that protein for the chick than does a similar assay conducted on an acid hydrolysate, as the latter values tend to be high. Clandinin and Robblee (1952) indicated that when a series of meals, processed under similar circumstances, are hydrolysed enzymatically, the amino acid contents of the hydrolysates as measured by the microbiological technique tend to indicate the nutritive value of the meals. If amino acids liberated by acid hydrolysis and measured by the microbiological method are not necessarily available to the chick what are the chances of riboflavin not liberated by acid hydrolysis being utilised by the suckling pig?

Davis *et al.* (1951) used neither acid nor an enzymatic hydrolysis on sows' milk prior to assaying for riboflavin by the microbiological method. Using a culture of *Lactobacillus casei* (E) they obtained levels which are generally accepted as being measures of the total riboflavin content of the milk. Braude *et al.*, as reported by Davis *et al.* (1950), assayed riboflavin in a similar manner to Davis *et al.* (1951) and also used the method of Kornberg *et al.* (1948) the latter method making use of bacteria of the species *Leuconostoc mesenteroides*. As the values obtained utilising *L. casei* were higher than those obtained using *Leuc. mesenteroides*, this could indicate that whereas all the riboflavin is available to one species of bacteria it is not necessarily available to all others. Even if the

total riboflavin of sows' milk is available to bacteria prior to hydrolysis this still does not prove that the same quantity of riboflavin would be available to the young pig. Before the value of measurements of the riboflavin content of sows' milk can be adequately assessed the availability of this vitamin in the milk must be determined.

Summary

1. It would appear that the two pregnancy rations fed contained an excess of vitamin A or provitamin A for no difference was noted in the vitamin A levels of the milk of sows from differing farrowing periods.
2. A measure of the 'acid liberated riboflavin' content of sows' milk is reported and a significant difference noted between the levels found in milk samples collected at different periods of the year.

THE VITAMIN A CONTENT OF THE BLOOD PLASMA OF SOWS AND THEIR SUCKLING PIGS

Objective

The measurement of the vitamin A content of sows' milk is of little value unless the volume of milk produced is known. However if the vitamin A level of the blood of the sow influences the amount excreted in the milk then there should be some relationship between the blood plasma A level of sows and their suckling pigs for the level in the plasma is dependent to a large extent upon dietary intake. It was in an effort to determine such a relationship that the following experiment was conducted.

Experimental

The sows which were selected for milk studies were also used in this experiment. The young pigs from these litters were ear notched soon after birth and the male and female piglets bearing the lowest numbers in each litter were used in the experiment.

A sample of approximately 15 ml. of blood was drawn from the anterior vena cava of each animal by means of a hypodermic syringe using the method devised by Carle and Dewhirst (1942). The blood was placed in a tube, containing two or three drops of a saturated potassium oxalate solution,

which was then stoppered and inverted several times. On arrival at the laboratory the blood was centrifuged and the plasma was assayed for vitamin A as described in the Appendix.

Results

The mean values obtained for the vitamin A content of sows' plasma are reported in table 8 while those for their piglets are reported in table 9. As an analysis of variance indicated that there was no difference in plasma vitamin A levels between male and female piglets, the means of both sexes have been combined. The results are also shown as graphs on page 54.

Table 8

Vitamin A in the Blood Plasma of Sows* (µ/100 ml.)

Date of Farrowing	Day after farrowing						
	7th	14th	21st	28th	35th	42nd	49th
January	35.2			22.2			22.5
Feb.-March	28.6	38.0		37.2			39.8
July	49.2	41.5	36.1	33.9	47.2	41.5	41.0
September	49.8	39.9	35.8	40.9	48.3	35.2	36.3

* There were four sows in each farrowing period.

Figure 4

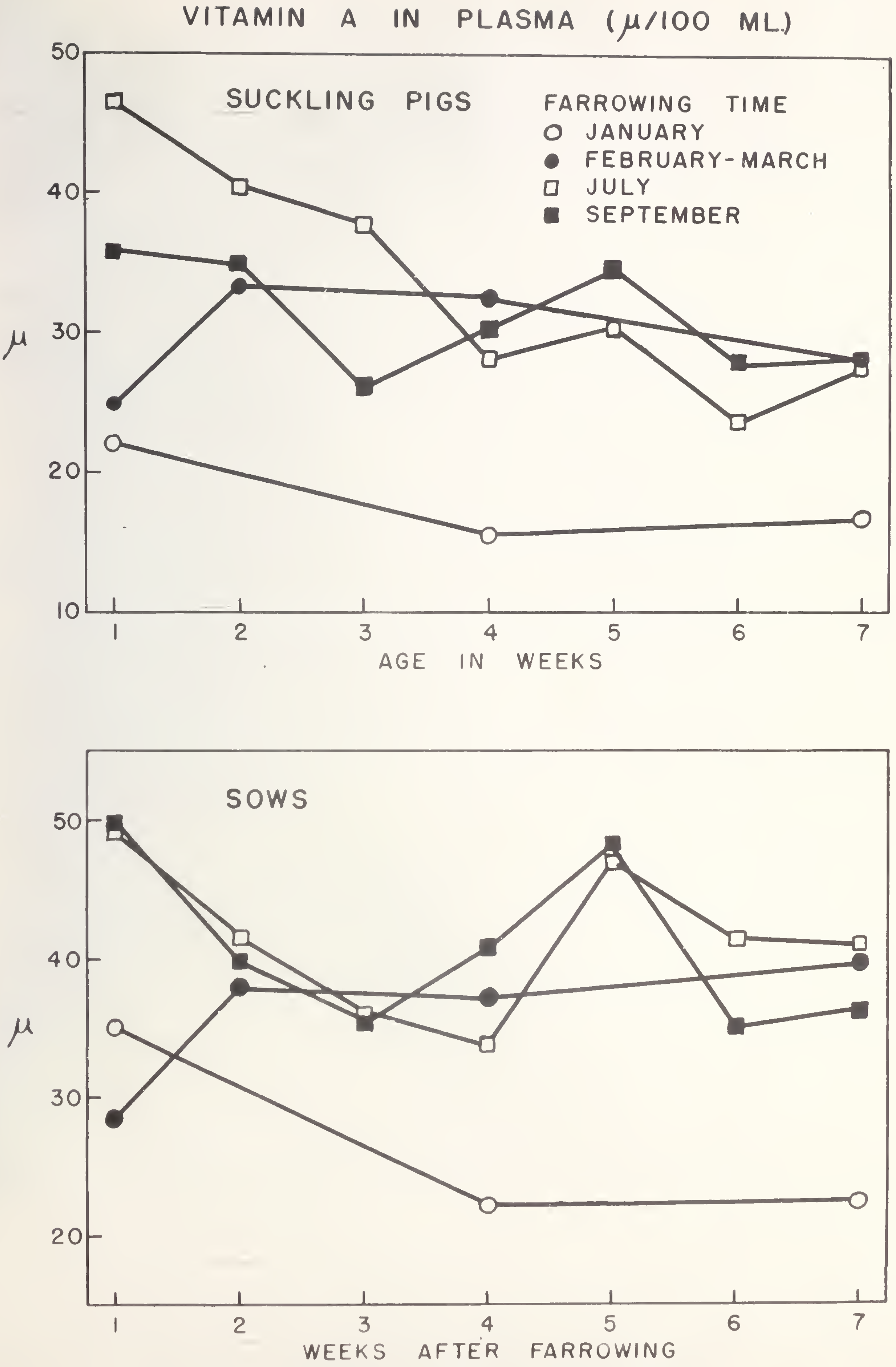

VITAMIN A IN PLASMA (μ/100 ML.)
SUCKLING PIGS
FARROWING TIME
○ JANUARY
● FEBRUARY-MARCH
□ JULY
■ SEPTEMBER
μ
50
40
30
20
10
1
2
3
4
5
6
7
AGE IN WEEKS
SOWS
μ
50
40
30
20
1
2
3
4
5
6
7
WEEKS AFTER FARROWING

Table 9

Vitamin A in Blood Plasma of Young Pigs* (μ/100 ml.)

Date born	Age in days 7	14	21	28	35	42	49
January	22.1			15.6			16.8
Feb.-March	24.9	33.4		32.5			28.1
July	46.5	40.5	37.9	28.2	30.4	23.6	27.4
September	35.9	35.0	26.2	30.3	34.6	27.9	28.0

* Each mean is representative of eight pigs.

The raw data obtained from the assay of vitamin A in the blood plasma of the pigs under test was submitted to a series of statistical analyses with the following results:

A simple correlation between the level of vitamin A in the blood plasma of sows and the mean level for one male and one female pig from each litter yielded the following data. Age of the young pigs and the stage of lactation of the sows were neglected.

	D.F.	r obtained	5%	1%
January	10	0.691	0.576	0.708
Feb.-March	14	0.621	0.497	0.623
July	26	0.461	0.374	0.478
September	26	0.573	0.374	0.478

These values indicate a significant positive correlation between the vitamin A content of the blood plasma of the

sow and the blood plasma A content of her young. The significance of the positive correlation indicated that further analysis would be justified. This further analysis took the form of a variation of the analysis of covariance, details of the analysis being as on page 57.

From the analysis and assuming a normal curve distribution, it is possible to formulate a series of regression equations from which we may predict the vitamin A content of the blood plasma of suckling pigs assuming that we know the level of this vitamin in the blood plasma of their dams.

Using the following notations:

$\ddot{Y}$ is the predicted level of vitamin A in the blood plasma of the young pigs,

$\bar{y}$ is the mean value for all young pigs measured in this experiment,

b_{yx} is the regression coefficient,

X is the level of vitamin A in the blood plasma of the dam of the young pigs for which the prediction is to be made,

$\bar{x}$ is the mean value for all sows measured in this experiment,

then:-

$$\ddot{Y} - y = b_{yx}(X - \bar{x})$$

$$\ddot{Y} = b_{yx}X - b_{yx}\bar{x} + \bar{y}$$

$$\ddot{Y} = b_{yx}X + C$$

where:-

$$C = (- b_{yx}\bar{x} + \bar{y})$$

Table 10 gives all the data necessary for inclusion in such an equation.

Week	D.F.	Sums of Squares and products			Correlation coefficient	Regression coefficient	Errors of estimate Sums of squares	
		SS_x	S_{xy}	SS_y	$\frac{S_{xy}}{\sqrt{(SS_x)(SS_y)}}$	$\frac{S_{xy}}{SS_x}$	$SS_y - \frac{(S_{xy})^2}{SS_x}$	D.F.
1	31	8199.99	4990.66	6383.33	0.690	0.609	3345.93	30
4	31	4815.39	2276.01	2410.24	0.668	0.473	1200.24	30
7	31	5384.95	1838.12	2028.28	0.556	0.341	1400.85	30
Sum	93	18400.23	9104.79	10821.85			5947.02	90
Total	95	19377.69	10002.80	11765.80				
Weeks	2	977.37	898.01	943.95				
Within weeks	93	18400.32	9104.79	10821.85	0.645	0.495	6316.65	92

	Error of estimate					
Source of variation	D.F.	S.S.	M.S.	F.	5%	1%
Deviation from average regression within lots	92	6316.65				
Deviation from individual lot regression	90	5081.26	66.08			
Difference among lot regression	2	1235.39	617.70	9.35	3.10	4.85

Table 10

Statistics Used in the Prediction of the Vitamin A Content of the Plasma of Suckling Pigs

			μA/100 ml.					
Week	b_{yx}	s_{byx}*	$\bar{x}$	$\bar{y}$	s_y**	s_x***	$s_{y \cdot x}$****	$\ddot{Y}$
1	0.609	0.119	40.7	32.4	14.35	16.26	10.56	0.609X+ 7.6
4	0.473	0.093	33.6	26.7	8.82	12.46	6.33	0.473X+10.8
7	0.341	0.095	34.9	25.1	8.09	13.18	6.83	0.341X+23.0
1 - 7	0.495	0.066	36.4	28.1	10.78	14.07	8.92	0.495X+18.4

* s_{byx} is the standard error for the regression coefficient (b_{yx}).

** s_y is the standard deviation for Y.

*** s_x is the standard deviation for X.

**** $s_{y \cdot x}$ is the standard deviation for Y when X is given.

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

Discussion

The above results indicate that the vitamin A level of the blood plasma of suckling pigs is dependent upon the level of this factor in the plasma of their dams. It has been shown by Lewis et al. (1941, 1942), Braun (1945), Henry et al. (1949), Sobel and Rosenberg (1950) and Merrow et al. (1952) that the vitamin A content of the blood plasma is influenced by the dietary intake of vitamin A or carotene. Therefore if we feed the sow an adequate quantity of this vitamin or its precursor we can ensure a relatively high level of A in the blood plasma which in turn will ensure a relatively high level in the plasma of her suckling pigs.

Examination of table 8 indicates that a measurement of the vitamin A level in the sow's blood plasma is useful in predicting the level of vitamin A in the blood plasma of her pigs. It would appear that the most accurate prediction can be made when the young pigs are four weeks of age for at this point the standard error of the regression coefficient (s_{byx}) and the standard deviation for Y when X is known ($s_{y \cdot x}$) are both at a minimum. The reason for this is probably that the liver stores of vitamin A with which the young pigs are born are still influencing the blood plasma levels of this vitamin at one week of age and consequently there is a much greater variation of Y. When

the pigs are seven weeks old they will be consuming creep feed which contains vitamin A, consequently they are no longer dependent upon the sow for provision of this factor. At four weeks of age the liver stores will have ceased to be a major influence and the sows milk will be the only source of vitamin A consequently at this point one would expect a greater relationship between the blood plasma levels of vitamin A in the sow and her young. A measure of the vitamin A content of sows' milk can never assure us that the suckling pigs are receiving an adequate supply of this vitamin unless we have a fairly accurate measurement of the milk production of the sow and milk consumption of her young.

A regression equation for the prediction of the blood plasma levels of vitamin A in suckling pigs between 1 and 7 weeks of age, assuming a knowledge of the level of this factor in the blood plasma of the dam, is also indicated in table 10. However little faith may be placed in this calculation as an analysis of variance indicated significant differences ($P = < 0.01$) between the regression coefficients of the first, fourth and seventh weeks.

The above analysis may be criticised on the basis of the small amount of data available. However it is felt that a true analysis has been given and that the errors due to the small sample have been adequately expressed. Although the accuracy of the predictions is limited due to the size

of sample and therefore the size of the errors (s_{byx} and $s_{y \cdot x}$) it is felt that this is a line of research which, if expanded over a larger population, would yield valuable results. By utilising a larger population the size of the errors (s_{byx} and $s_{y \cdot x}$) would be decreased and hence the accuracy of the prediction would be increased.

As there was no expression of vitamin A deficiency in the suckling pigs, used in this experiment, it is not possible, from the results in table 9 or from the raw data from which this table was calculated, to estimate a level below which the vitamin A in the blood plasma of the sow should not be allowed to fall. The lowest vitamin A level reached by any sow in this experiment was 8.3µ/100 ml. of plasma but this was only for a short period. It would seem advisable however to attempt to maintain a level well in excess of this value. Hentges _et al._ (1952) noted that visible deficiency symptoms did not occur until the blood plasma A level fell below 5µ/100 ml. plasma.

Summary

1. Data are presented to show that the vitamin A content of the blood plasma of suckling pigs bears a highly significant positive correlation to the level in the plasma of their dams.
2. A regression equation has been formulated from which a prediction of the vitamin A level in the blood

plasma of suckling pigs may be made if the level of this vitamin in the blood plasma of their dam is known.

3. No significant difference in the blood plasma vitamin A level of male and female suckling pigs of the same age from the same litter was observed.

HAEMOGLOBIN

Experimental

During 1954 the haemoglobin content of the blood of one male and one female pig from each litter born was measured at one week of age and then at fourteen day intervals until the pigs were nine weeks old. This is a useful measurement for not only can it indicate the onset of anaemia, but it may give a reflection of the general health of the animals, as many other diseases affect the haemoglobin level of the blood. The method of measuring the haemoglobin is reported in the Appendix.

The administration of reduced iron to the piglets is a routine treatment. All pigs born in January received 0.5 to 1.0 gm. of such iron, mixed with feeding oil, at 3, 10, 17 and 24 days of age. All pigs farrowed at subsequent periods during 1954 received similar doses of reduced iron though the frequency of administration was increased, the iron being given to the pigs when they were 3, 7, 10, 14, 17, 20 and 24 days of age.

Results

The mean values obtained from the measurement of haemoglobin in the blood of male and female pigs are given in table 11. There was no difference between the levels

in the blood of males and females. A series of graphs indicating the various haemoglobin levels at different farrowing periods will be found on page 65.

Table 11

The Haemoglobin Content of the Blood of Young Pigs (gms./100 ml.)

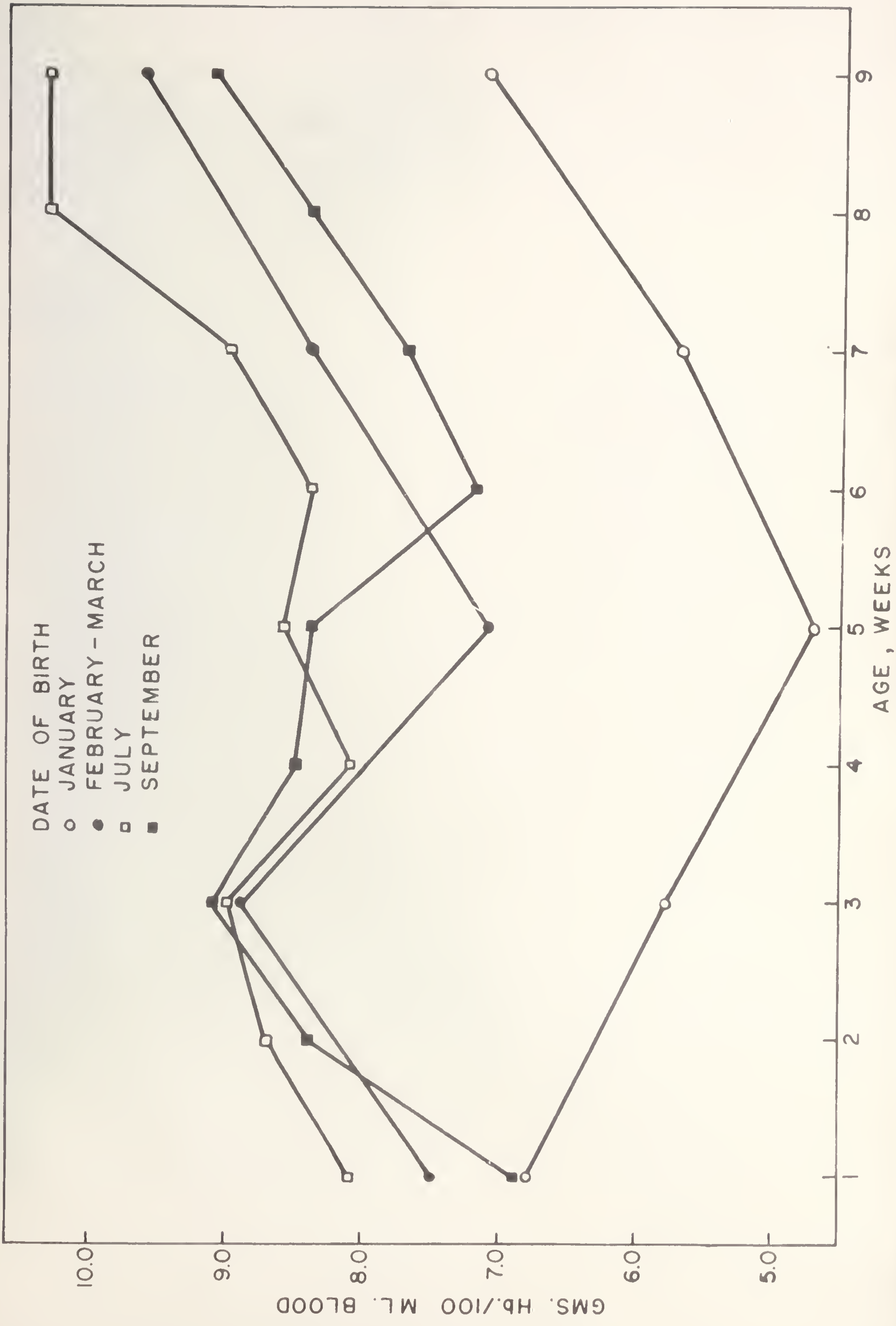
Born	Age of pigs in days				
	7	21	35	49	63
January	6.8	5.8	4.7	5.7	7.1
Feb.-March	7.5	8.9	7.1	8.3	9.6
July	8.1	8.6	8.6	9.1	10.3
September	6.9	9.1	8.4	7.7	9.1
Mean	7.3	8.1	7.2	7.7	9.0

An analysis of variance of the data collected is given below.

Analysis of Variance

	D.F.	S.S.	M.S.	F.	5%
Farrowing	3	483.98	161.33	2.41	2.62
Age	4	211.80	52.95	0.79	
F x A	12	826.18	68.85	1.03	
Error	465	31,178.45	67.05		
Total	484	32,700.41			

Figure 5


HAEMOGLOBIN LEVELS
DATE OF BIRTH
○ JANUARY
● FEBRUARY-MARCH
□ JULY
■ SEPTEMBER
GMS. HB./100 ML. BLOOD
10.0
9.0
8.0
7.0
6.0
5.0
AGE, WEEKS
1
2
3
4
5
6
7
8
9

This analysis would indicate that at the 5% level of significance there was no difference between the haemoglobin levels of the pigs born in different farrowing periods nor was there any apparent difference in the haemoglobin levels of pigs at different ages.

Discussion

The data reported in this section were collected in order that an estimation of the health of the young pigs involved in the creep feed experiment, reported earlier, could be made. Noting that a number of the pigs born in January, 1954 had blood haemoglobin levels approaching that of clinical anaemia, considered to be 3.5 gm./100 ml. blood (Hamilton, 1933), it was decided that pigs born subsequent to this period should be given reduced iron more frequently. It should be pointed out that as all these pigs were being used in a creep feed experiment it was not possible to divide the young pigs into several different groups which could then be subjected to different iron treatments.

As will be noted from the graphs on page 65 the pigs born in January had a considerably lower average blood haemoglobin level than pigs born at other times of the year. An analysis of variance however, indicated that there was no significant difference between the blood haemoglobin levels of pigs farrowed in January and pigs

farrowed later in the year. This lack of significance is probably due to the considerable variation of the blood haemoglobin levels within groups.

Since the blood haemoglobin levels of pigs born in January were not significantly different from those of pigs born at other periods of the year, even though the latter received considerably more reduced iron, the value of reduced iron for the prevention of anaemia in young pigs may be questioned. One would imagine that a source of iron, suitable for the prevention of anaemia in pigs, would not only raise the average blood haemoglobin level when administered more frequently, assuming the level was not already at a maximum, but would also tend to raise the blood haemoglobin level of every pig in such a way that the within group variation would be reduced. Similar doubts as to the usefulness of reduced iron in the prevention of anaemia in young pigs have been expressed verbally by Dr. L. E. Hanson of the University of Minnesota and Dr. A. J. Wood of the University of British Columbia.

Although the writer has certain doubts as to the effectiveness of reduced iron in the prevention of anaemia in young pigs, it should be pointed out that the variation in the above data could have been due to seasonal variations. However comparison of the January and February-March born pigs in respect to birth weight, weaning weight and creep feed consumption would indicate that the

variation observed between the average blood haemoglobin levels of these two groups was probably due to the frequency of the administration of reduced iron.

Summary

1. It is not possible to draw definite conclusions from the haemoglobin data collected during 1954 as the reduced iron administration treatments were confounded with seasons.
2. Pigs receiving reduced iron at 3, 10, 17 and 24 days of age had an average haemoglobin level which was considerably lower than that of pigs receiving iron at 3, 7, 10, 14, 17, 20 and 24 days of age.
3. An analysis of variance indicated that there was no significant difference between treatments, probably due to the considerable variation within groups.

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APPENDIX

1. Vitamin A in Sows' Milk.*

Reagents: Potassium hydroxide solution - 20 gm. KOH + 10 ml. water + 90 ml. ethyl alcohol.

Acidified alcoholic wash solution - 1 ml. HCl + 100 ml. ethyl alcohol + 1 litre of water.

Procedure:

a. To 5 ml. of colostrum and 15 ml. water or 10 ml. milk and 10 ml. water, add 30 ml. of potassium hydroxide solution and let stand in a separatory funnel (A) overnight in the dark.

b. Add 60 ml. of water.

c. Add 25 ml. ether. Shake for 1 minute. Remove the lower layer to a second separatory flask (B) and add 20 ml. of ether to this. Shake 1 minute. Discard the lower layer.

d. Add 10 ml. of acidified wash solution to (A). Invert 4 times letting vapors escape. Transfer the lower layer to a second flask (B) and do likewise. Discard the lower layer.

e. Add 2 ml. petroleum ether to (A) and to (B).

f. Repeat the washing step (d) twice more.

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* Adapted from the method of Boyer, P. D., R. Spitzer, C. Jensen and P. H. Phillips. 1944. Determination of vitamin A and carotene in milk. A rapid extraction procedure. Ind. Eng. Chem., Anal. Ed., 16: 101-102; as modified by Bowland *et al.* (1949b).

g. Combine the ether in flasks (A) and (B) and allow to stand 15 minutes. Remove the wash solution settling to the bottom.

h. Draw off the ether into a graduate cylinder and pipette a 2/5 aliquot into the colorimeter tube.

i. Follow, starting at step f in the procedure for vitamin A determination in the blood.

Calculations

Milk Log of the galvanometer reading x 332.5 equals micrograms of vitamin A per 100 ml. milk.

Colostrum Log of the galvanometer reading x 665 equals micrograms of vitamin A per 100 ml. colostrum.

2. 'Acid Liberated Riboflavin' in Sows' Milk.

a. To 25 ml. of milk add 12 ml. of 0.2 N H_2SO_4 and autoclave at 15 lbs. for 15 minutes.

b. Cool and adjust to pH 6.0 with 1 N NaOH. Since riboflavin is unstable in alkaline solution the extract should be swirled constantly during the addition of alkali to prevent local areas of high pH. Immediately add 1 N H_2SO_4 to bring to pH 4.5.

c. Dilute the solution to 100 ml. with water and filter.

d. To a 50 ml. aliquot of the filtrate add 1 N H_2SO_4 dropwise until no more precipitate forms. Follow by an approximately equal number of drops of 1 N NaOH, with constant shaking. Dilute to 100 ml. and filter again if necessary.

e. Place 10 ml. of the filtrate from d + 1 ml. of water + 1 ml. of glacial acetic acid in a clean cuvette and measure the riboflavin content fluorometrically.*

f. A Coleman Model 14 Universal Spectrophotometer with fluorometric attachments and appropriate filters was used to measure the fluorescence. Sodium fluoroscein solution gave standard fluorescence while blanks were determined by adding 20 mg. of sodium hydrosulphite to each of the riboflavin containing cuvettes and remeasuring their fluorescence.

3. Vitamin A in Blood Plasma.**

a. Samples are centrifuged for from 5 to 10 minutes.

b. To a series of test tubes add 5 ml. of 95% ethyl alcohol, 14 ml. of petroleum ether and 5 ml. of plasma.

c. Stopper the tubes and shake for 5 minutes.

d. Set the tubes in the dark for two to three hours.

e. Pipette 10 ml. of supernatant petroleum ether into a colorimeter tube.

f. Evaporate to dryness under a vacuum at 50°C.

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* Due to the fact that it was possible to recover in excess of 98% of added riboflavin by this procedure oxidation with 3% $KMnO_4$ followed by 3% H_2O_2 was considered unnecessary.

** Kimble, M. S. 1939. The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. Clin. Med. 24: 1055.

g. To all tubes, blank included, add 1 drop of acetic anhydride and 1 ml. of chloroform.

h. Put filter 620 mμ. in place in the colorimeter and turn on the current.

i. To the blank, add 9 ml. of antimony trichloride and set the galvanometer so that it reads 100. Determine the air blank and hold this blank constant, by necessary adjustments until all determinations are run.

j. Wipe unknown tube and insert into colorimeter.

k. Add rapidly 9 ml. of antimony trichloride solution and read the galvanometer at the first stationary point. ($SbCl_3$ soln. is made by adding 100 gm. of $SbCl_3$ to 500 ml. chloroform).

Micrograms vitamin A per 100 ml. are calculated using the formula: Log of G reading - (.2051 x L_{440}) x 372.4 where L_{440} = log of the carotene reading and 372.4 and .2051 are conversion factors.

4. Hemoglobin Determination* (Oxyhemoglobin method)

a. To clean colorimeter tubes add 10 ml. distilled water and stopper.

b. Add .02 ml. (blood pipette) of blood, obtained from an ear vein, and shake.

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* Evelyn, K. A. 1936. A stabilised photoelectric colorimeter with light filters. J. Biol. Chem. 115: 63-75.

c. To a blank (10 ml. distilled water) and to determination tubes add 1 drop of concentrated ammonium hydroxide.

d. Using an Evelyn colorimeter with filter 540 $m\mu$. in place set the blank at 100. Determine air blank and hold constant.

e. Obtain readings on the blood and calculate grams of hemoglobin per 100 ml. of blood as follows:

$$\frac{100 \times L_{540}}{2.58}$$

L_{540} is the log of the galvanometer reading when using filter 540 $m\mu$.

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